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ORIGINAL ARTICLE

Environmental factors affecting chytrid (Chytridiomycota) infection rates on *Planktothrix agardhii*

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Planktothrix agardhii dominates the cyanobacterial harmful algal bloom biomass in Sandusky Bay, Lake Erie (USA) from May until September. This filamentous cyanobacterium known parasites including the chytrid fungal species *Rhizophydium* sp. C02, which was previously isolated from this region. The purpose of our work has been to establish how parasitic interactions affect *Planktothrix* population dynamics during a bloom event. Samples analyzed from the 2015 to 2019 bloom seasons using quantitative PCR investigate the spatial and temporal prevalence of chytrid infections. Abiotic factors examined in lab include manipulating temperature (17–31°C), conductivity (0.226–1.225 mS/cm) and turbulence. *Planktothrix*-specific chytrids are present throughout the bloom period and are occasionally at high enough densities to exert parasitic pressure on their hosts. Temperatures above 27.1°C in lab can inhibit chytrid infection, indicating the presence of a possible upper thermal refuge for the host. Data suggest that chytrids can survive conductivity spikes in lab at levels three-fold above Sandusky Bay waters if given sufficient time (7–12 days), whereas increased turbulence in lab severely inhibits chytrid infections, perhaps due to disruption of chemical signaling. Overall, these data provide insights into the environmental conditions that inhibit chytrid infections during *Planktothrix*-dominated blooms in temperate waters.

KEYWORDS: *Planktothrix*; cyanobacteria; chytrid; harmful algal bloom

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INTRODUCTION

Planktothrix harmful algal blooms are a global issue (Kurmayer *et al.*, 2016), affecting major water bodies across Europe (e.g. France [Jacquet *et al.*, 2005]; Poland [Mankiewicz-Boczek *et al.*, 2011; Bukowska *et al.*, 2017]; and the Nordic countries [Rohrlack *et al.*, 2009]); North America (Kutovaya *et al.*, 2012; Steffen *et al.*, 2014; Davis *et al.*, 2015; McKay *et al.*, 2018) and Asia (Ueno *et al.*, 1996; Lin *et al.*, 2010). Indeed, *Planktothrix agardhii* is the dominant cyanobacterial species in the annual toxigenic cyanobacterial harmful algal blooms (cHABs) that occur in Sandusky Bay, Lake Erie (Rinta-Kanto and Wilhelm, 2006; Davis *et al.*, 2015; Salk *et al.*, 2018). It is a species that is competitive at low light intensities, having the capability to control its buoyancy by utilizing gas vesicles (Walsby and Klemer, 1974; Walsby *et al.*, 1983; Beard *et al.*, 2000; Becker *et al.*, 2005; Walsby, 2005; Carey *et al.*, 2012), promoting success in the shallow and turbid waters of environments like Sandusky Bay.

Chytridiomycota—informally chytrids—is the classification of a group of fungal organisms based on their ability to produce zoospores encased in sporangia as part of their life cycle (Canter, 1967). Within this classification is the order *Rhizophydiales*, which encompasses parasitic chytrids that are known to infect a number of phytoplankton species (Canter and Lund, 1951), including *Planktothrix* (Sønstebø and Rohrlack, 2011; Rohrlack *et al.*, 2013; Kyle *et al.*, 2015; Rohrlack *et al.*, 2015; McKindles *et al.*, 2021). *Planktothrix*-specific chytrids have been previously isolated from two Nordic water bodies (Sønstebø and Rohrlack, 2011) and Sandusky Bay (McKindles *et al.*, 2021).

Isolation of both *P. agardhii* hosts and their *Rhizophydiales* chytrid pathogens from Sandusky Bay occurred in 2018, allowing for a closer analysis of host–parasite interactions in the lab (McKindles *et al.*, 2021). The chytrid isolates from Sandusky Bay are closely related to the chytrid isolate *Rhizophydiales* chy-kol2008 (Sønstebø and Rohrlack, 2011) based on ITS-rRNA sequencing, morphology, life cycle and host specificity (McKindles *et al.*, 2021). These isolates display different infection rates, likely due to differences in physiological traits of zoospore release and host specificity, or susceptibility to antifungal compounds produced by specific hosts (McKindles *et al.*, 2021). Other studies have shown that these chytrid pathogens are affected by host nutrient ratios (Frenken *et al.*, 2017) and by secondary metabolites produced by their hosts (Rohrlack *et al.*, 2013).

Although *Planktothrix*-specific chytrids have been isolated from bloom events, enumerating chytrids and determining host–pathogen relationships through environmental sampling has yet to be broadly investigated. The best example of characterizing the presence of these pathogens in a bloom setting is the work by Rohrlack *et al.* (2015), where the authors utilized qPCR to construct a relationship between chytrid presence and the diversification of host chemotypes over a 13-year time period. Further, they characterized a lower thermal refuge where the host can grow but chytrid infections are not sustained, and no longer serving as a source of parasitic (top-down) pressure. Around the same time, Kyle *et al.* (2015) utilized a similar approach to characterize host–parasite presence in sediment cores dating back to the start of the 1980s. This work revealed that chytrids may favor one *Planktothrix* sp. chemotype over another.

This study aims to further our understanding of the environmental factors that affect chytrid infections during *Planktothrix*-dominated blooms in Sandusky Bay (Lake Erie, Laurentian Great Lakes), and to characterize the prevalence of infection in a water body distinct from those previously studied. Here, we quantitate chytrid and host presence from 2015 to 2019 through qPCR methods and compare total presence through abundance of gene copy numbers mL^{-1} across four sites and across 5 years. In addition to qPCR analysis, data were obtained to determine the role of physiochemical parameters influencing chytrid infections on a susceptible host, specifically temperature, turbulence and conductivity. As a shallow lacustrine estuary, Sandusky Bay tends to be significantly affected to high flow events and river suspended sediment inputs (Conroy *et al.*, 2017), which likely correlate with spikes in turbulence and conductivity as nutrients are delivered to the bay from the Sandusky River. Environmental data were then combined with lab experiments to relate chytrid infections in context with real world infection parameters.

MATERIALS AND METHODS

Sampling sites and water processing

At the southeast corner of the western basin of Lake Erie is the shallow (mean depth 2.6 m) Sandusky Bay (Fig. 1), which is divided into the outer bay (eastern half) and the inner bay (western half). With assistance from research vessels and crews of the Ohio Department of Natural Resources Watercraft Division, four sites were monitored as part of this work: ODNR4, the Edison Bridge, ODNR1 and EC1163. Microbial biomass (50–240 mL) was collected in the field onto a 0.22 μm Sterivex cartridge, which was immediately stored on dry ice. Once in the lab, the filters were moved to a −80°C freezer until the DNA could be extracted. Samples were collected every 2 weeks at a constant depth of 1-m depth from the surface at three sites (ODNR4, ODNR1 and EC1163)

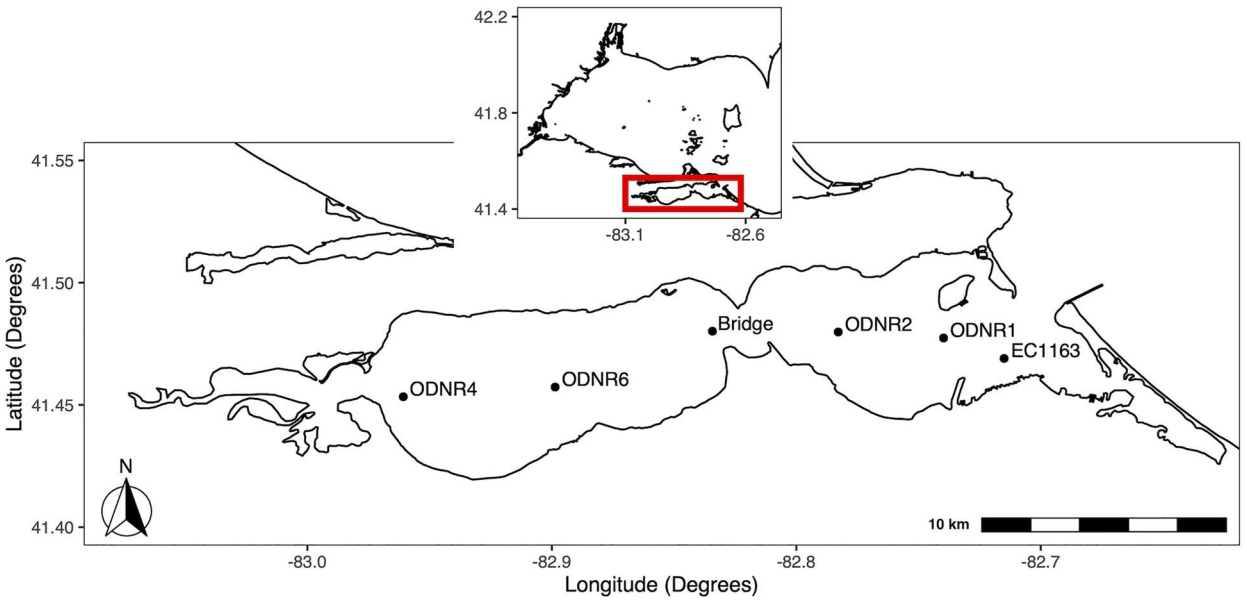

Fig. 1. Map of sampling locations in Sandusky Bay. The inset shows the location of Sandusky Bay in relation to the western basin of Lake Erie. Sites were chosen for a cross-section of the width and depth of the bay. Sites were ODNR4 (41.453333 and −82.960767), Edison Bridge (41.480156 and −82.834328), ODNR1 (41.477367 and −82.739783) and EC1163 (41.469000 and −82.715000).

during the bloom season (May–September) of 2015–2019, and an additional fourth site (Bridge) in 2018 and 2019. Further samples were also collected just above the sediment at two sites (ODNR4 and ODNR1) in 2019.

In addition to sampling for planktonic biomass, physicochemical data were measured at approximately the same time for each site during each sampling trip using a calibrated model 600 QS water quality probe (YSI Inc., Yellow Springs, OH, USA) to measure surface water temperature, dissolved oxygen concentration and conductivity. Light transmission in the water column was measured with a Li-COR submersible quantum sensor (Lincoln, NE, USA). Wind speeds were downloaded from the Great Lakes Observing System Data Export Tool (glbuoys.glos.us) from the bgsusd2 Sandusky Bay buoy for the 2017–2019 sampling seasons. Wind speed data were unavailable for the 2015–2016 sampling seasons.

Monitoring of chytrid presence by quantitative PCR

DNA was extracted from individual Sterivex samples using the DNeasy PowerWater Sterivex kit (Qiagen, Germantown, MD, USA) per the manufacturer's instructions, and stored post extraction at −80°C until used for qPCR analysis. DNA quantity was checked using a Quantus Fluorometer (Promega, Madison, WI) and the associated QuantiFluor ONE dsDNA System kit (Promega, Madison, WI), per manufacturer's instructions.

To quantify the presence of the host, *P. agardhii*, species specific primers rpoC1_Plank_F271 and rpoC1_P_agardhii_R472 were used (Churro *et al.*, 2012; Table I). To quantify *Planktothrix*-specific chytrids in Sandusky Bay, a 2013 metagenome data set from Sandusky Bay (SRX1991078) was downloaded and the files were imported into CLC Genomics Workbench v.12.0.2 (Qiagen, Redwood City, CA, USA) with the default quality settings following Steffen *et al.* (2017). Failed reads were discarded during import. Paired-end reads for both samples in the metagenome data set were trimmed for quality prior to being combined for assembly into contigs (minimum length 2000 bp) using the CLC Genomics Workbench *de novo* assembly function that also mapped reads back to the generated contigs. The generated contigs were then tested against a BLAST search using the chytrid Kol-2008 and the chytrid Lys-2009 ITS sequence (FR670787 and FR670788).

The contig matching the chytrid sequences was used to design primers for local *Planktothrix*-specific chytrids (Table I) by adding the contig to the primer designer web application (OligoPerfect Primer Designer; Thermo Fisher Scientific, Waltham, MA, USA). Selected primer sets were then analyzed by BLAST against the metagenomic data set to assess possible spurious hybridization. Once tested, the region inclusive of the primer sets and extra base pairs in either direction was extracted to create external standards (Table II). External standards were used to determine copy numbers of each qPCR target by creating 10-fold dilution series of G-block gene

Table I: qPCR primers for Planktothrix agardhii and Planktothrix-specific chytrids

Target	Forward (5′–3′)	Reverse (5′–3′)	Product size (bp)	Primer efficiency	Reference
P. agardhii rpoC1	TGTTAAATCCAGG TAACTATGACGGCCTA	GCGTTTTTGTCC CTTAGCAACGG	224	0.971	Churro *et al*. 2012
Chytrid ITS	GCTCCTCGTT GAGCTCACTT	GTATCGCATTTC GCTGCGTT	216	0.915	This study

Table II: qPCR g-block sequences used to generate standard curves

Target	Sequence (5′–3′)	Size	Standard Curve Range (copies μL^{-1})
P. agardhii rpoC1	ATGTGGTGTTAAATCCAGGTAAC TATGACGGCCTATCCTATAAACAG TTATTAACCGAAGATACTTGGTTAG AAATTGAAGACCAAATTTATAGTGA AGATTCCACCTTAACCGGAATTGAAG TGGGAATTGGAGCCGAAGCCATTTCCC GTTTGCTCGAAGATATTCCCTTAGAAGA AGAAGCCGAAAGATTACGGGAAGAAAT TGCCGTTGCTAAGGGACAAAAACGCGCCAAA	240	3.866×10^9–38.66
Rhizophydium sp. ITS	TTTTAAGTTGATGCTCCTCGTTGAGCTCACTTG ATTCAACTCCCTTTTCACACTTTGTGCACTATG ATTGTTTTTTGGGTTGACTGTTACCCATTGGCG ACGTCAACCCAGCATTATTTAAAACCATTGTTA ATTTGTCTGAATTTTACATATAGTAAATTAAAAA CAACTTTTGACAACGGATCTCTTGGTTCTCGCA ACGATGAAGAACGCAGCGAAATGCGATACGTA ATGTGAATT	236	3.802×10^9–38.02

fragments (Integrated DNA Technologies, Coralville, IA, USA). G-blocks were diluted to 10-ng μL^{-1} stocks, and the total copy number of G-block fragments was calculated using the formula: number of copies (molecules) = (A ng $\times$ 6.0221 $\times$ 10^{23} molecules $mole^{-1}$)/([N $\times$ 660 g $mole^{-1}$] $\times$ 1 $\times$ 10^9 ng g^{-1}), where A is the amount of amplicon in ng, N is the length of the dsDNA amplicon and 660 g $mole^{-1}$ is the average mass of 1 bp dsDNA (Prediger, 2013). The range of the *P. agardhii* specific *rpoC*1 standard was 3.866×10^9–38.66 copies μL^{-1} and the range for the *Rhizophydium* sp. ITS standard was 3.802×10^9–38.02 copies μL^{-1} (Table II). Positive controls for qPCR primers included isolated and purified host and chytrid (McKindles *et al*., 2021), and negative controls included non-*Planktothrix* cyanobacteria and uninfected samples. Note that this analysis is comparing a single-copy gene for the host and a multi-copy gene for the parasite. Nonetheless, relationships between chytrid and host presence is dependent on encystment on filament termini, which can average 125 cells per filament in culture (Reichwaldt and Abrusan, 2007; Vergalli *et al*., 2020). Consequently, one may conclude that a 1% qPCR relationship between the two indicates an active infection.

Real-time PCR was performed using 5 µL of each extracted DNA with the PowerUp SYBR Green Master Mix (Thermo Fisher Scientific) and 400 nM of each primer (Table I). Each sample was run under the same conditions multiple times using the different primer sets, as each reaction was a singleplex run. After an initial activation step at 50°C for 2 min and a denaturing step at 95°C for 2 min, 40 cycles were performed as follows: 15 s at 95°C, 30 s at 55°C and 60 s at 72°C. A melt curve was also performed to ensure a single qPCR product was formed, going from 50 to 95°C at an increase of 0.5°C per cycle. The program was run on a 4-channel Q Real-Time PCR thermocycler (Quantabio, Beverly, MA, USA) along with the Q-qPCR v1.0.1 software analysis program (Quantabio), which was used to determine the sample concentrations as compared with a standard curve. The efficiency of the rpoC1 primer set is 0.971 and the efficiency of the chytrid ITS primer set is 0.915 (Table I).

Physicochemical effects on infection rates

Environmental effects were assessed using the host *P. agardhii* 1031, a microcystin-producing strain (Dm-MC RR and Dm-MC LR) isolated from Sandusky Bay in 2016 by Dr Rainer Kurmayer (Universität Innsbruck). The culture was maintained in unialgal, non-axenic batch culture in Jaworski's Medium (JM; Culture Collection

of Algae and Protozoa) in 125 mL glass flasks at 22°C. Light was supplied by warm-white fluorescent tubes at a light–dark cycle of 12 h:12 h delivering a photosynthetic photon flux density (PPFD) of 10 µmol photons m^{-2} s^{-1}. For infections, mid-exponential phase cultures (~8000 filaments mL^{-1}) of *P. agardhii* 1031 were infected with *Rhizophidium* sp. isolate C02 at a 1:10 v/v dilution (~2% infected filaments final abundance). *Rhizophidium* sp. isolate C02 was maintained as described in McKindles *et al*. (2021).

Experimental samples were investigated for incidence of infection as a proportion of infected filaments in the population. Similar to previous work (McKindles *et al*., 2021), there were no noticeable differences in the intensity of infection for each treatment (number of mature sporangia per filament; see Results below) and infections were lethal regardless of the number of sporangia on a filament. The abundance of infected and uninfected *P. agardhii* was counted under an inverted microscope at a magnification of ×400. Replicate samples were fixed with glutaraldehyde (0.5% final concentration) and were counted in duplicate (a minimum of 60 filaments each) using a hemocytometer.

Conductivity

Conductivity was tested by monitoring infections in six different conductivity environments (0.226, 0.426, 0.622, 0.826, 1.023 and 1.225 mS/cm) in six replicate infections each. The conductivity of standard JM was measured using a model 600 QS sonde (YSI) and was modified by adding 0.1 M NaCl to standard media at ~0.2-mS/cm intervals from 0.226 to 1.225 mS/cm, the upper limit of which matches the conductivity of the frequently used BG-11 freshwater media. *Planktothrix agardhii* 1031 was inoculated at ~1000 filaments mL^{-1} in the modified media and allowed to acclimate for 7 days. After 7 days, acclimated cultures were standardized to 8000 filaments mL^{-1} prior to the introduction of *Rhizophidium* sp. isolate C02 at a 1:10 v/v dilution at 22°C with a light–dark cycle of 12 h:12 h delivering a PPFD of 10-µmol photons m^{-2} s^{-1}. Experiments were conducted in 2.0 mL volumes in non-treated 24-well plates (USA scientific, Ocala, FL, USA). Samples were removed from the six replicate infections every 2 days and were monitored for a period of 12 days before the experiment was terminated.

Mixing effects

Mixing effects on infection prevalence was measured by shaking infected cultures at seven shaking speeds (0, 50, 57, 65, 73, 96 and 120 rpm) in four replicate infections each. *Rhizophidium* sp. isolate C02 was inoculated on a fresh culture of *P. agardhii* 1031. The experiment was conducted in 50-mL Erlenmeyer culture flasks filled to a maximum volume of 15 mL. Infected cultures were placed in the same standing incubator (Caron model 7304-50-2, Marietta, OH, USA) at 22°C with a light–dark cycle of 12 h:12 h delivering a PPFD of 10 µmol photons m^{-2} s^{-1}. One set of samples was placed on a shaking table (VWR shaker model 35 000, Radnor, PA, USA) set to 50, 57, 65, 73, 96 or 120 rpm while the control set was left on a stationary rack. Samples were pulled from the replicate flasks at 2-day intervals and were monitored for a period of 9 days before the experiment was terminated.

Mixing speeds were converted to turbulent kinetic energy (TKE) dissipation rate (ε), which is the parameter most widely used to characterize turbulence in biological systems, using equation (19) taken from Guadayol *et al*. (2009). The equation was $\log_{10}(\varepsilon) = -5.03 - 1.56\varphi + (1.71 + 1.08\varphi) \times F$, where φ is the orbital diameter in cm and F is the frequency (s^{-1}) of the orbit.

Water temperature

The effects of temperature on chytrid infections were monitored using a temperature gradient chamber (Supplemental Fig. 1) as described by Watras *et al*. (1982). In brief, an aluminum bar with a heating element controlled by an Inkbird PID temperature controller thermostat ITC-106 (Inkbird, Shenzhen, China) at one end and an ActiveAqua model AACH10HP water chiller (Petaluma, CA, USA) set to 4°C at the other end was used to create a stable temperature gradient (17.1, 18.6, 20.2, 21.7, 23.1, 24.3, 25.9, 27.1, 28.9 and 30.1°C). The temperature gradient bar is designed to accept triplicate 50 mL conical tubes at each temperature totaling 30 tube across the 10 temperatures. Light was supplied from below using a system that was identical to the lighting in the incubator where the stocks were stored. Each experiment was incubated at 10 µmol photons m^{-2} s^{-1} from cool fluorescent lamps at 12:12 h light:dark cycles. To prevent a thermal gradient from developing, the tubes contained a maximum volume of 15 mL. To rule out any temperature shock for the hosts at the outset of the experiment, diluted replicate cultures of *P. agardhii* 1031 were placed at each temperature and allowed to acclimate for 2 weeks. After host acclimation, acclimated cultures were standardized to 8000 filaments mL^{-1} and *Rhizophidium* sp. isolate C02 was added to each replicate at 1:10 v/v dilution. Samples were removed from the replicate tubes at 2-day intervals and were monitored for a period of 13 days before the experiment was terminated.

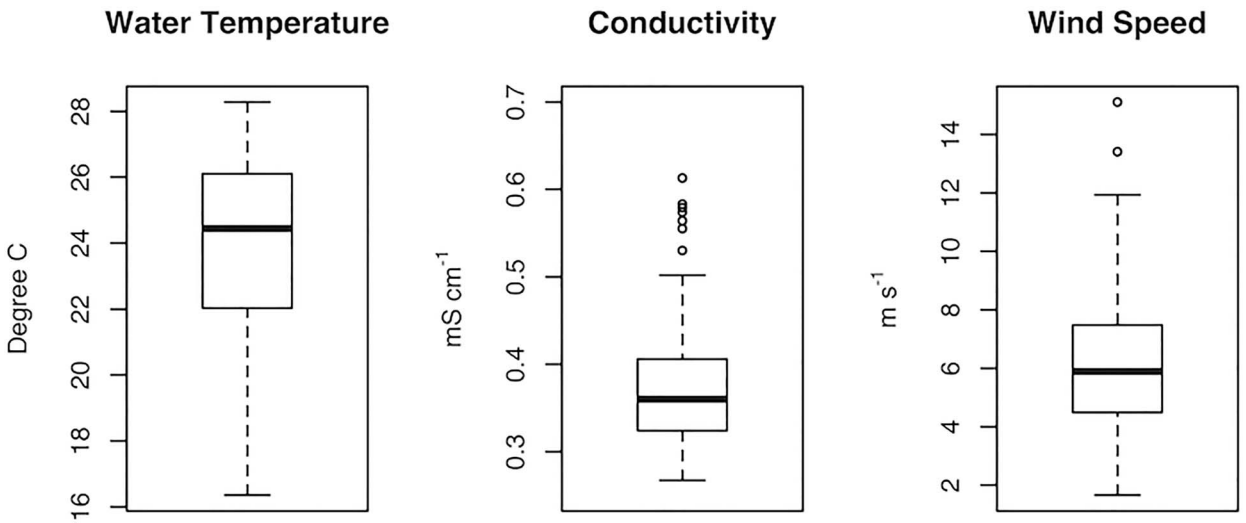

Fig. 2. Measured abiotic parameters of Sandusky Bay sites ODNR4, Edison Bridge, ODNR1 and EC1163 from 2015 to 2019. Samples were acquired every 2 weeks during the bloom season (May–September) when available. Each individual measurement was combined to generate boxplots that represent seasonal averages and extremes. Wind speeds were only measured 2017–2019. Raw values for abiotic parameters can be found in Supplementary Tables 1 and 3.

Statistics

Statistics for trends and regression analyses were performed using RStudio version 1.0.153 working on R version 3.6.1 (2019-07-05). Statistical differences between abiotic factors (temperature and conductivity) across years were determined individually by one-way ANOVA analysis followed by TukeyHSD multiple comparison test, where sites were consolidated. Statistical differences between qPCR gene copy numbers between sites and across years was determined by two-way ANOVA analysis followed by TukeyHSD multiple comparison test. Statistical differences between the infection percentage at the water's surface and the water-sediment interface was determined by a two-way ANOVA across sites and location within the water column. All analysis of variance (ANOVA) results are summarized in Supplemental Table 4.

Models were generated for each experimental effect using the stats v3.6.2 package in R. The conductivity model was created using the function SSasymp, the mixing effects model was created using the function SSlogis and the water temperature model was created using the poly function.

RESULTS

Physiochemical data of Sandusky Bay

For each of the four sites (ODNR4, Edison Bridge, ODNR1, EC1163; Fig. 1), physicochemical data obtained from the water quality sonde during field seasons 2015–2019 were combined to generate a range of conductivity values and water temperatures that could be used to correlate relationships between environmental parameters with infection patterns in Sandusky Bay. During the sampling seasons (May–September, 2015–2019) in Sandusky Bay, the average conductivity was 0.375 ± 0.075 mS cm^{-1} and the average water temperature was 24 ± 2.6°C (Fig. 2). At the extremes, seasonal water temperatures ranged from 16.4 to 28.3°C and conductivity measures ranged from 0.267 to 0.613 mS cm^{-1} (Supplemental Table 1; Bullerjahn and McKay, 2020a,b). Sampling during 2016 revealed warmer water temperatures and higher conductivity readings (26.0 ± 1.4°C and 0.423 ± 0.109 mS cm^{-1}; $P < 0.01$; Supplemental Table 1), but there were no significant variations in either parameter between 2015 and 2017–2019 (TukeyHSD post hoc $P > 0.05$, Supplemental Table 4 A and B). In addition, data were downloaded from the Great Lakes Observing System Data Export Tool (Supplemental Table 3) for wind speeds on Sandusky Bay for the 2017–2019 sampling seasons, which averaged 6.08 ± 2.03 m s^{-1}.

Monitoring chytrid presence by quantitative PCR

Chytrid and host presence was monitored over 5 years to determine the prevalence of infection in the wild. Host abundances were quantified using the single copy housekeeping gene *rpoC*1 (Fig. 3A), with values ranging from

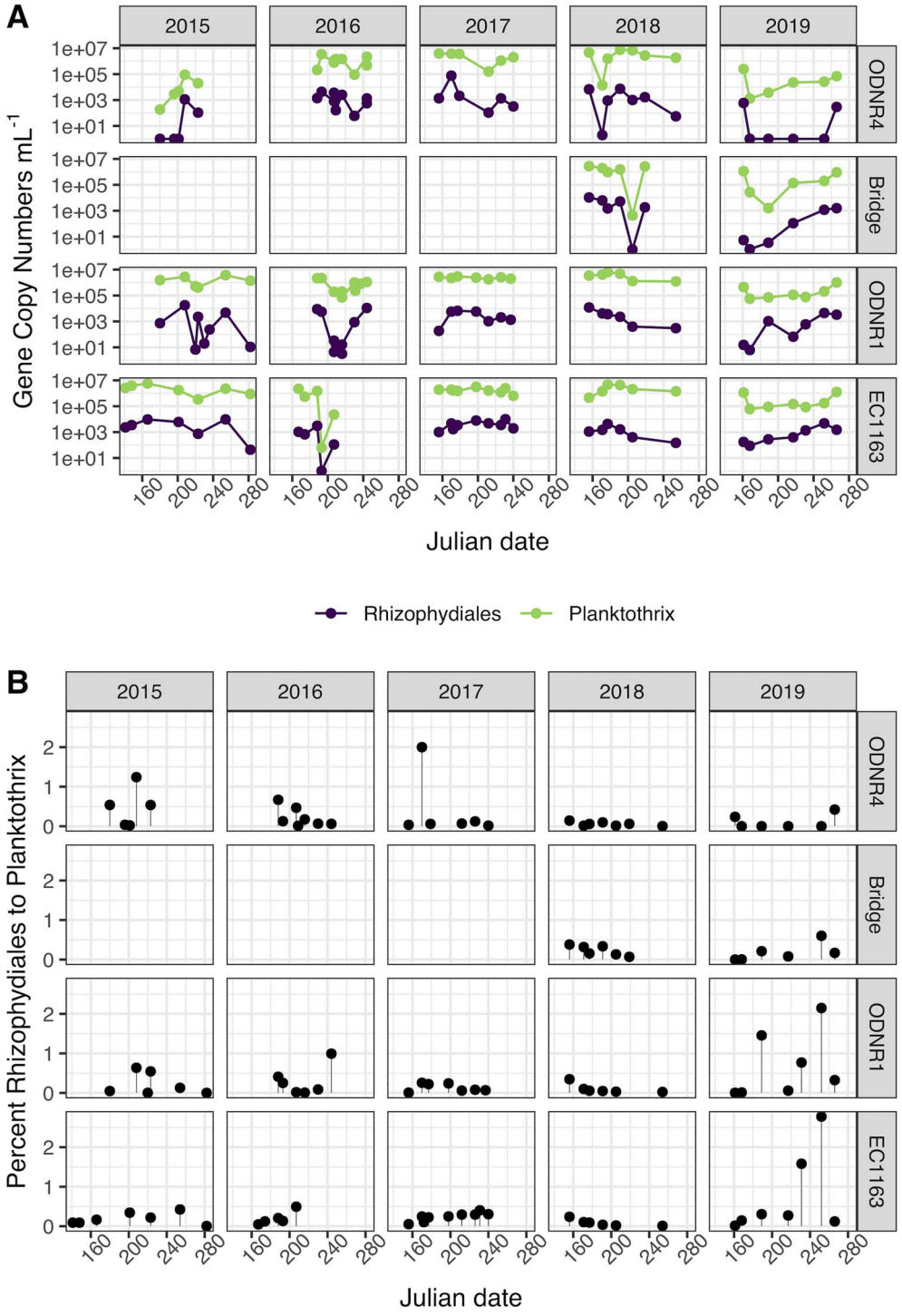

Fig. 3. qPCR quantitation of *Planktothix agardhii* and *Planktothrix*-specific chytrid at sites ODNR4, Edison Bridge, ODNR1 and EC1163 over 5 years (2015–2019). (**A**) *Planktothrix agardhii* is quantified using *P. agardhii* specific primers for the rpoC1 gene, and *Planktothix*-specific chytrids are quantified based on *Rhizophydiales* sp. primers specific for Sandusky Bay isolates (McKindles *et al.*, 2021). (**B**) The relationship between host and parasite as a percent of chytrid DNA over host DNA.

62.0 (EC1163 on 11 July 2016) to 7.62E+06 (ODNR4 on 10 July 2018) gene copies mL^{-1} over the 5-year period. Despite the high level of interannual variation (early bloom, mid-bloom, late bloom), there were statistically significant differences between host abundances in 2018 compared with 2015 ($P < 0.005$), 2016 ($P < 0.001$) and 2019 ($P < 0.001$), suggesting that 2015, 2016 and 2019 were less intense blooms than 2018 in terms of *Planktothrix* biomass during sampling periods (Supplemental Fig. 2 and Supplemental Table 4C).

Planktothrix-specific chytrids were quantified using primers designed from the multi-copy ITS-rDNA28 sequence, with values ranging from < 10 (lowest positive hit, but below the quantification limit, at the Bridge site on 17 June 2019) to 7.61E+04 (ODNR4 on 19 June 2017) gene copies mL^{-1} over the 5-year period (Fig. 3A). Unlike host abundance, chytrid abundance did not vary across years and between sites (TukeyHSD post hoc $P > 0.05$, Supplemental Table 4D), suggesting a more constant presence in the bay. Chytrid abundance tended to mirror host abundance as decreases in chytrid gene copy numbers correlated with declines in host gene abundance as observed at EC1163 in 2016, and Bridge and ODNR4 in 2018 (Fig. 3A).

Since both the chytrid and the host were quantified in each sample, we were able to estimate the percent of chytrid abundance relative to the host. Although present most of the time, chytrid infections were rare (Fig. 3B). Chytrid gene abundance compared with the host gene abundance over the sampling period averaged $0.27 \pm 0.45\%$, ranging from a low of 4.74E−04% (Bridge on 10 June 2019) to a high of 2.78% (EC1163 on 9 September 2019). Whereas a majority of the values were <1%, 6 dates (one in 2015, one in 2017 and four in 2019) showed elevated infection percentages (Fig. 3B). As above, there were no statistical differences in infection percentages across years and between sites (TukeyHSD post hoc $P > 0.05$, Supplemental Table 4E).

Additional sampling was performed in 2019 at two sites (ODNR1 and ODNR4) in the water column just above the sediment to determine if there were differences in host and chytrid abundance depending on location within the water column. The average abundance at each site for both the chytrid and its host were higher at the sediment interface compared with the water surface (Fig. 4). Variance in the average abundance at either location was due to date-by-date differences. When each date was taken as a single point, the abundance of both genes was higher at the sediment compared with the surface, ranging from a 11.6 to 642.5 times greater abundance of *P. agardhii* at ODNR1 and a 3.2–38.2 times greater abundance at ODNR4. Further, although chytrid genes were only found on one date at ODNR4 at the sediment,

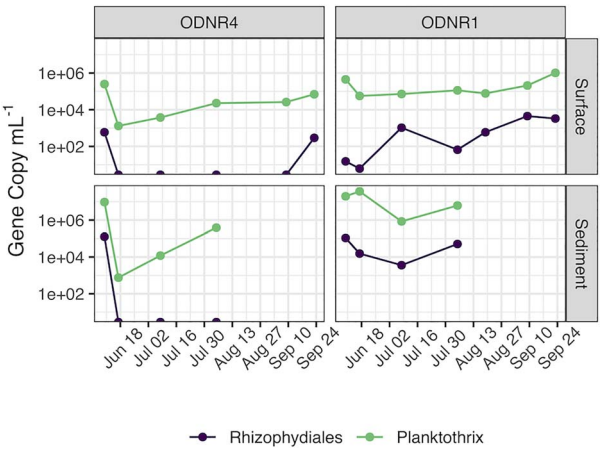

Fig. 4. qPCR quantitation of *Planktothix agardhii* and *Planktothrix*-specific chytrid at sites ODNR4 and ODNR1 in 2019 at the water's surface (<1-m depth) and just above the sediment.

chytrid abundance at ODNR1 ranged from 3.5 to 7079.2 times greater at the sediment compared with the surface. Despite the increase in biovolume towards the sediment for both host and chytrid, infection percentages, ranging from 0.05 to 2.15%, were similar between the bottom and surface samples and across sites (two-way ANOVA; $P > 0.05$, Supplemental Table 4F).

Conductivity

Increasing conductivity induced fewer chytrid infections (Fig. 5). In our experiments, $81.1 \pm 12.3\%$ of filaments became infected by chytrids by Day 12 in unmodified Jaworski's Medium (JM; 0.226 mS/cm). This declined to an average of $37.5 \pm 6.2\%$ infected filaments in the 0.426–1.023 mS/cm JM modified media, and further dropped to $9.1 \pm 4.1\%$ infected filaments in the 1.225 mS/cm JM (Fig. 5A). Initial effects (Days 0–7) of the different conductivity strengths are immediate; the percent increase in infected filaments per day for unmodified JM is 12.0%, whereas the modified media range from increases of 0–1.7%, averaging $0.9 \pm 0.6\%$ increase in infected filaments per day (Fig. 5B). Finally, the chytrids appear to recover in the modified media after 7 days, but infection percentages still remain lower than the unmodified media: the 0.426–1.023 mS/cm JM had an increase in infected filaments per day of $6.2 \pm 0.8\%$, whereas the 1.225 mS/cm JM only had an increase of $1.6 \pm 0.8\%$ (Fig. 5A).

Mixing effects

As an organism that requires attachment to a particular location on a host as part of their lifecycle, constant

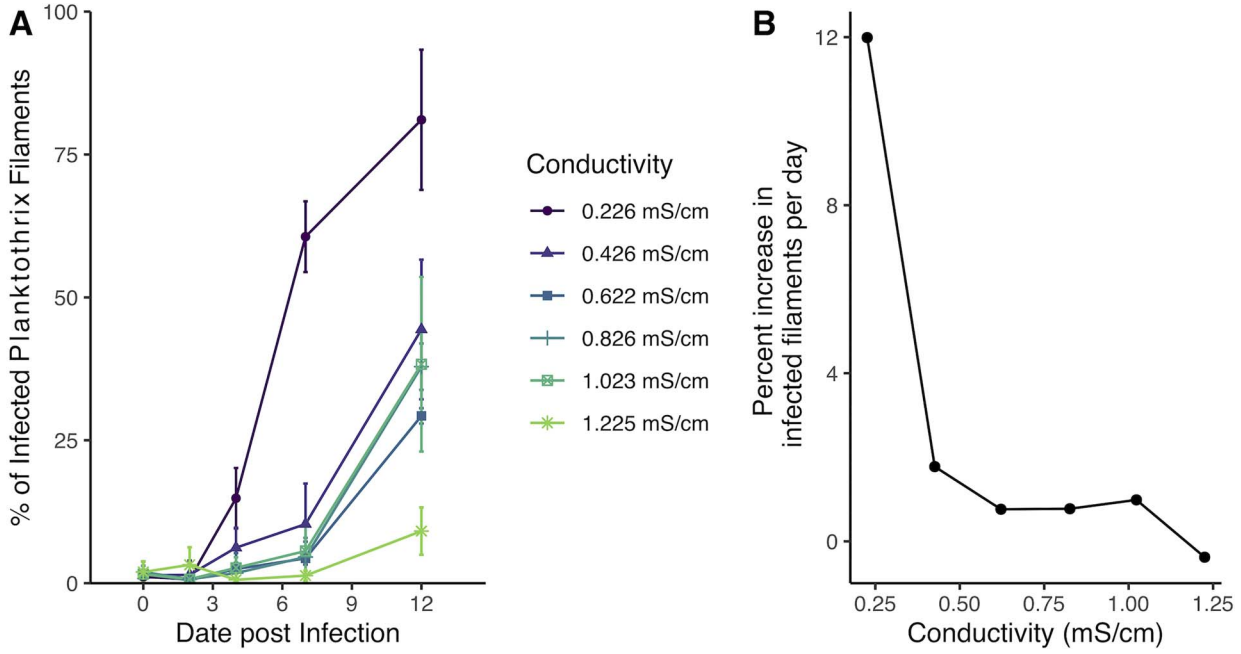

Fig. 5. The effect of increasing conductivity (mS cm^{-1}) on infection rates. Conductivity values range from standard Jaworski's media (0.226 mS cm^{-1}) to standard BG-11 media (1.225 mS cm^{-1}) (**A**) Lower conductivity media shows increased percentage of infected *Planktothrix agardhii* filaments. (**B**) Rates (day^{-1}) of infection percentage of *P. agardhii* filaments until Day 7 of experiment. Error bars indicate standard deviation.

agitation may cause significant inhibition of infection prevalence. Mixing speeds on an orbital shaker can be converted to turbulence kinetic energy (TKE or ε; Guadayol *et al.*, 2009). In our experimental setup, no shaking equated to a $\log10(\varepsilon)$ of -10.284 m^2s^{-3}. With shaking, the minimum turbulence tested was $\log10(\varepsilon) = -8.57$ m^2s^{-3} (50 rpm) and the maximum turbulence tested was $\log10(\varepsilon) = -6.01$ m^2s^{-3} (125 rpm). Successful infections occurred in stationary ($\log10(\varepsilon) = -10.284$ m^2s^{-3}) or low turbulence samples ($\log10(\varepsilon) = -8.57$ and -8.33 m^2s^{-3}), but the chytrids could not establish themselves at greater turbulence speeds ($\log10(\varepsilon) > -8.06$ m^2s^{-3}; Fig. 6A). Further, at greater turbulence speeds, infection rates were close to zero, indicating that new infections were not occurring (Fig. 6B).

Water temperature

Chytrid infections occur most frequently in a temperature range of starting with the lowest tested temperature of 17.1°C up to 24.3°C, with an optimal temperature range of 21–22°C (Fig. 7). Host growth is also affected by changes in temperature. Although *P. agardhii* 1031 grows well at the lowest tested temperature of 17.1°C up to 27.1°C, the strain has an optimal growth temperature of 23°C and grows poorly above 28°C (Supplemental Fig. 3). At temperatures between 17.1 and 24.3°C, >70% of the filaments in culture are infected, and as temperature increases from 24.3°C, the maximum percentage of infected filaments greatly decrease; 64.1% at 25.9°C, 32.0% at 27.1°C, 12.1% at 28.9°C and 8.3% at 30.1°C (Fig. 7A). Even within the ideal temperature range for these chytrid infections, there is a difference in infection rate, where the highest rate of infections occurred at 21.7 and 17.1°C, and while the same maximum infected filament percentage was reached, infections occurred more slowly at 18.6, 20.2, 23.1 and 24.3°C (Fig. 7B).

DISCUSSION

The work described here used laboratory experiments to characterize the effect of several important physicochemical parameters that are found in our model system (Sandusky Bay) on infections by chytrid fungal pathogens (*Rhizophidium* sp. isolate C02) and their host (*P. agardhii*). Sandusky Bay (Fig. 1) is a shallow basin (mean depth of 2.6 m) with warm surface water temperatures (23.95 ± 2.55°C), moderate conductivity (0.375 ± 0.075 mS cm^{-1}) and moderate average wind speeds (6.08 ± 2.03 m s^{-1}; Fig. 2). Conductivity measurements in Sandusky Bay compare well with that measured in Scandinavian lakes where Chytridiomycota are present

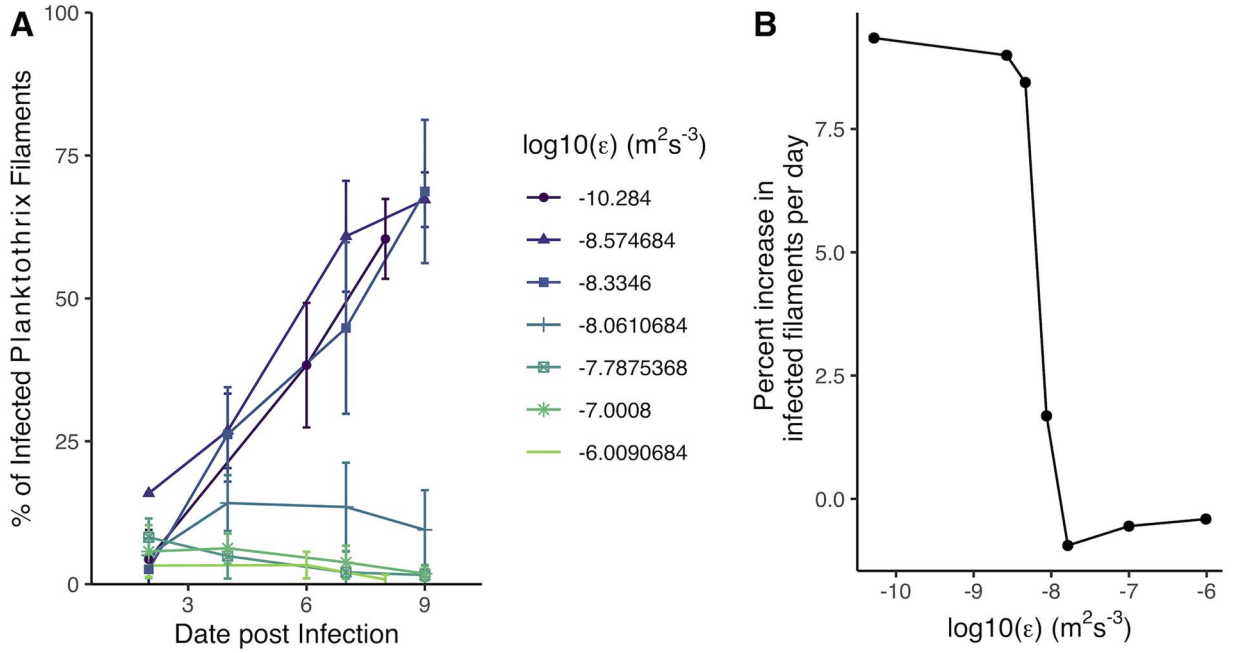

Fig. 6. The effect of increasing turbulence as a function of TKE on infection rates. Minimum turbulence was $\log10(\varepsilon) = -8.57\ m^2s^{-3}$ (50 rpm) and maximum turbulence was $\log10(\varepsilon) = -6.01\ m^2s^{-3}$ (125 rpm). (**A**) Lower turbulent kinetic energy (slower shaking speeds) shows increased percentage of infected *Planktothrix agardhii* filaments. (**B**) Rates (day^{-1}) of infection percentage of *P. agardhii* filaments until Day 9 of experiment or specific curve reached plateau. Error bars indicate standard deviation.

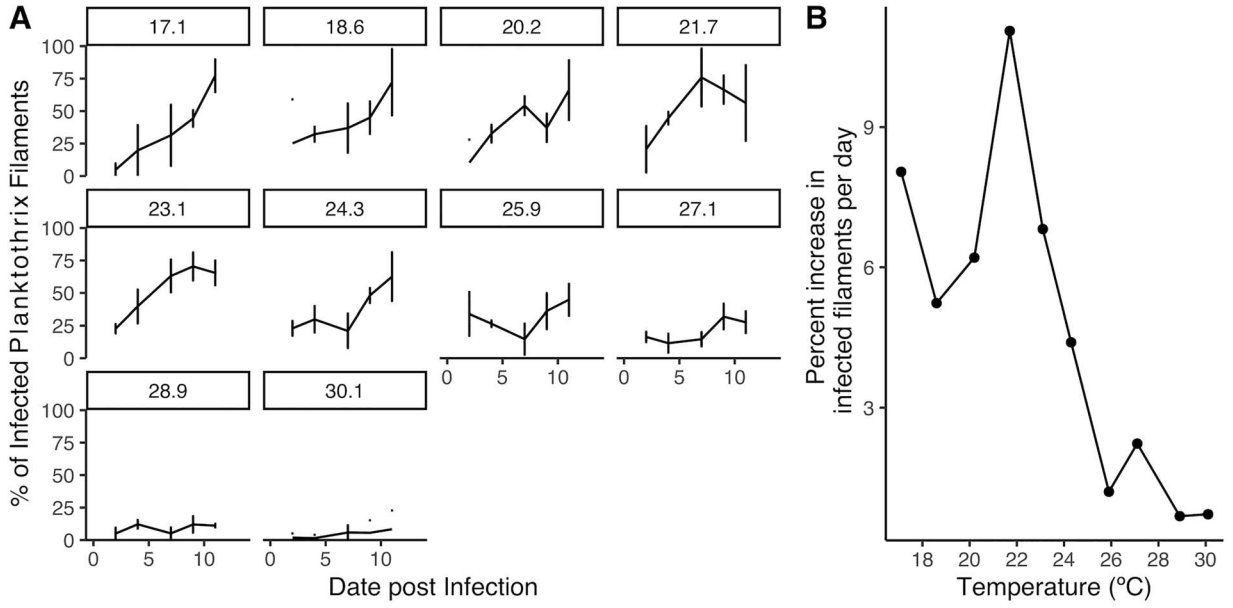

Fig. 7. The effect of increasing temperature (°C) on infection rates. Chytrid infections occur most frequently at an optimal temperature of 21–22°C. (**A**) Higher temperatures show decreased percentage of infected *Planktothrix agardhii* filaments. (**B**) Rates (day^{-1}) of infection percentage of *P. agardhii* filaments until Day 12 of experiment or specific curve reached plateau. Error bars indicate standard deviation.

(0.441 ± 0.336 mS cm^{-1}), and more specifically where *Rhizophydiales* sp. can be found (Khomich *et al.*, 2017). Alternatively, mean surface water temperatures were considerably higher in Sandusky Bay than the Norwegian lakes from which *Rhizophydiales* sp. were previously isolated (Lake Hålandsvatnet at 8.47°C and Lake Kolbotnvannet

at 6.3°C), whereas wind speeds were only slightly higher (Lake Hålandsvatnet at 4.59 m s^{-1}, and Lake Kolbotnvannet at 2.58 m s^{-1}; Rohrlack *et al.*, 2015). The differences between these water bodies suggests a wide ecological range at which *Rhizophydiales* sp. can exist, as long as their host is present.

Sandusky Bay was monitored for the presence of *Planktothrix*-specific *Rhizophydiales* sp. over the course of 5 years, which showed that chytrids are constantly present but are not in high abundance (Fig. 3). Rohrlack *et al.* (2015) found that historically *Planktothrix* in Lake Hålandsvatnet was likely under high parasitic pressure by chytrids through the calculation of a low ratio (less than 200) of host DNA to parasite DNA. In comparison, infection percentages of 0.5% (equivalent to a 200 parasitic pressure ratio) and greater occurred four times in 2015, two times in 2016, once in 2017 and six times in 2019 in Sandusky Bay (Fig. 3B). Without further genetic characterization of the host, we are unable to confirm that these points of parasitic pressure did or did not have an effect on the genetic diversity of the *Planktothrix* community, but additional work is planned to differentiate host chemotypes.

The addition of above-sediment sampling at two sites, ODNR1 and ODNR4, in 2019 led to the discovery that both *Planktothrix*-specific chytrids and *P. agardhii* are both more abundant near the sediment–water interface than at the water's surface (Fig. 4). Although Sandusky Bay is known to be turbid due to its shallow depth and frequent sediment resuspension (Hampel *et al.*, 2019), this difference in host gene copy numbers within the water column may be an indication that chytrid infections tend to occur deeper in the water column. *Planktothrix* filament aggregation has been observed in the lab in response to chytrid infection (McKindles *et al.*, 2021), possibly leading to an increased sinking velocity of the host in relation to increased particle size (Titman and Kilham, 1976). Chytrid infected filaments may be found at higher frequency deeper in the water column either due to the added weight of the maturing sporangia and/or the loss of buoyancy control in infected cells as the rhizoids invade terminal cells. Rhizoids of *Rhizophidium* sp. were found in partially decomposed *Planktothrix* filaments and were extended through multiple host cells (Sønstebø and Rohrlack, 2011) and chytrid-infected filaments have been observed to sink in culture (McKindles *et al.*, 2021).

Increases in conductivity of the infection media above the mean conductivity of Sandusky Bay had an immediate effect on the rate of chytrid infections on *P. agardhii*, but long-term effects were modest as the chytrids were able to survive higher conductivity levels over time (Fig. 5). Previous work had noted that the differences in conductivity between two the low conductivity freshwater cyanobacterial media JM and the higher conductivity freshwater cyanobacterial media BG-11 was enough to prevent infections of several *Rhizophidium* sp. isolates on three susceptible *P. agardhii* host strains for up to 4 weeks (McKindles *et al.*, 2021). Changes in environmental conditions are particularly important in zoospores as the absence of a cell wall (Sparrow, 1960; Taylor and Fuller, 1981; Dorward and Powell, 1983) means that changes in osmotic pressure and mechanical forces can easily damage the cell membrane. Overall, it has been suggested that chytrid zoospores appear to not be tolerant to changes in salinity (Gleason *et al.*, 2006; Stockwell *et al.*, 2015; Scholz *et al.*, 2017), but little is currently known about the ability of zoospores to survive conductivity changes when they are already in the process of sporangia encystment, as well as those zoospores protected from salinity spikes by the cell wall of the mature sporangia. In addition, these changes in osmotic gradients could have an effect on the chemotaxis that the zoospores utilize when finding hosts. It is known that zoospores respond to chemical gradients induced by a number of different compounds, including carbohydrates and amino acids (Held, 1974; Mitchell and Deacon, 1986; Moss *et al.*, 2008; Scholz *et al.*, 2017), which can be disrupted via changes in osmotic gradients. This interaction suggests that localized spikes in conductivity can lead to osmotic shock to the zoospores and disrupt the chemical signals used by zoospores to locate additional hosts, but that ultimately, *Rhizophidium* spp. are capable of surviving in varying freshwater conductivity conditions. Indeed, the relationship between conductivity spikes and infection prevalence could represent a lifting of top-down controls on *Planktothrix* blooms just after a rain and nutrient loading event, possibly leading to an even larger response of *Planktothrix* to the influx of nutrients.

Increased shaking (> 57 rpm or $\log10(\varepsilon) > -8.06$ m^2s^{-3}) as a proxy for turbulence was the most effective factor in preventing chytrid infections in culture (Fig. 6). This type of experiment has been used before in plankton studies to show that small-scale turbulence affects dinoflagellate growth and reduced parasitism in culture (Berdalet *et al.*, 2007; Guadayol *et al.*, 2009; Llaveria *et al.*, 2010). To put shaking speeds in context of turbulence as a function of turbulence kinetic energy (TKE or ε), Guadayol *et al.* (2009) generated a series of formulae relating the two variables. Although TKE of Sandusky Bay has not yet been measured, the TKE of the open waters of western Lake Erie has been determined. TKE rates from western Lake Erie were taken during the summer of 2008–2009 and ranged between −7.6 and −6.3 m^2s^{-3}, which was considered to be representative of the nearshore regions throughout the lake (Lin *et al.*, 2021) and which is higher than a majority of the shaking speeds tested here (Fig. 6B). Further, Lin *et al.* (2021) clarified that the nearshore regions of Lake Erie were energized

by wind events that quickly dampen and that most of the TKE flux was associated with the surface mixed layer. Given its size, the most important factors when calculating the hydrology of Sandusky Bay in terms of turbulence are wind wave shear stress, storm surges and seiches (Lee *et al.*, 1994). Wind speeds of approximately 7.7 m s^{-1} have been shown to stir up blooms in western Lake Erie (Wynne *et al.*, 2010), but as a shallower water body, it is expected that wind speeds greater than 4–6 m s^{-1} (George and Edwards, 1976; Lick *et al.*, 1994; Hunter *et al.*, 2008; Conroy *et al.*, 2011) are sufficient for mixing the water's surface layer in Sandusky Bay. At an average wind speed of 6.08 ± 2.03 m s^{-1} for the 2017–2019 sampling seasons (Fig. 2), the water column is thoroughly mixed for most of the season (Supplemental Table 2).

Chytrid infections are highly regulated by temperature, with optimal infections occurring around 21.7°C and declining infections occurring at temperatures greater than 27.1°C (Fig. 7). Indeed, this *Rhizophidium* sp. isolate had a lower optimal temperature than its host, *P. agardhii*, which peaks around 23.1°C (Supplemental Fig. 3) and is known to reach max growth at temperatures between 20 and 25°C (Post *et al.*, 1985; Sivonen, 1990; Oberhaus *et al.*, 2007). Based on the data shown here, these chytrids do not appear to be well adapted to the higher temperature ranges favored by the host, likely creating an upper thermal refuge in which infections are low or completely inhibited. The formation of upper thermal refuges may be more likely with these isolates because Sandusky Bay surface water temperatures are much higher than the Norwegian lakes in which lower thermal refuges have been previously studied (Rohrlack *et al.*, 2015). Although this is the first report of a *Planktothrix*-specific chytrid having an upper thermal refuge, chytrid infections on diatom hosts have been shown to have both a "cold" and "hot" thermal refuge of low or no infection (Gsell *et al.*, 2013). With average temperatures at the surface in Sandusky Bay reaching 24 ± 2.6°C during the bloom season (Fig. 2), it is likely that the surface is too warm for chytrid infections and infected filaments are more likely to be found at depth and cooler temperatures and/or cooler seasons. Further, surface water temperatures become too warm (>24.5°C) in July and August (Supplemental Tables 1 and 3), meaning chytrid infections are not temperature limited at the water's surface only early in the bloom season (May and June) or during bloom decline (September and October).

The focus of the experiments outlined here only address three environmental parameters that can influence infection, but other factors also play a role in limiting chytrid infections *in situ* such as nutrient availably (Frenken *et al.*, 2017), light distribution (Tao *et al.*, 2020), grazing (Agha *et al.*, 2016; Gerphagnon *et al.*, 2019; Frenken *et al.*, 2018, 2020; Sanchez Barranco *et al.*, 2020) and antifungal compounds (Pancrace *et al.*, 2017; McKindles *et al.*, 2021).

CONCLUSION

Sandusky Bay is a shallow, turbid water body supporting recurrent annual cHABs dominated by *P. agardhii*, which is host to the chytrid *Rhizophidium* sp. There is evidence to suggest that chytrid presence is constant in the bay, and under certain conditions, chytrids are abundant enough to likely cause parasitic pressure on their hosts. Although infections can be diminished via high surface water temperatures and temporary spikes in conductivity, the greatest limitation for widespread chytrid infections in Sandusky Bay may be the constant surface mixing of the bay and occasional sediment–water interface mixing and sediment resuspension from high flow and very high wind events. Future work will aim at modifying these parameters in mesocosm experiments to determine their effect on wild populations of both host and parasite.

SUPPLEMENTARY DATA

Supplementary data can be found at *Journal of Plankton Research* online.

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